

The Synthesis of a Substance allied to Adrenalin.

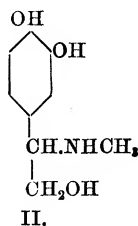
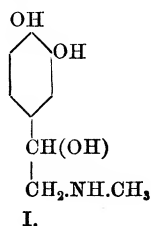
By H. D. DAKIN.

(Communicated by Professor E. H. Starling, F.R.S. Received May 29,—Read June 8, 1905.)

(From the Laboratory of Pathological Chemistry, Lister Institute of Preventive Medicine.)

The isolation of adrenalin from the suprarenal gland by Takamine (1) and by Aldrich (2) in 1901 has led to a considerable amount of work on the chemical nature of this remarkable substance. It is, however, only within the last two years that satisfactory elementary analyses and molecular weight determinations have been made, and the exact structural formula is still unsettled.

Two formulæ have been proposed, either of which will account for most of the observed facts concerning the chemical behaviour of adrenalin, although there are other possible structures which may demand consideration.*



A preliminary note has been published by Friedmann (5), which contains

* It is not proposed to give a *résumé* of previous work, but the following indisputable facts upon which speculations as to structure are based may be briefly stated—there is, however, much evidence of a less direct nature which will be found in the papers by Abel (3), von Fürth (4), Friedmann (5), Jowett (6), Pauly (7), and Stolz (8).

(a) Adrenalin, on fusion with alkali, gives catechol, so that an ortho-substituted benzene nucleus is present in the molecule.

(b) Adrenalin, on methylation and subsequent oxidation, gives veratric acid—

3:4 dimethoxybenzoic acid—pointing to the existence of the grouping $\text{—O} \begin{array}{c} \diagup \text{O} \diagdown \\ \text{C}_6\text{H}_3 \end{array} \text{.C} \begin{array}{c} \diagup \\ \diagdown \end{array}$; and since further adrenalin gives the colour reactions (FeCl_3 , etc.) of an ortho-dihydric phenol, it is concluded that two hydroxyl groups are present in the ring

$$\begin{array}{c}
 \text{HO} \\
 | \\
 \text{HO} \begin{array}{c} \diagup \text{C} \diagdown \\ \text{C}_6\text{H}_3 \end{array} \text{.C} \begin{array}{c} \diagup \\ \diagdown \end{array}
 \end{array}$$

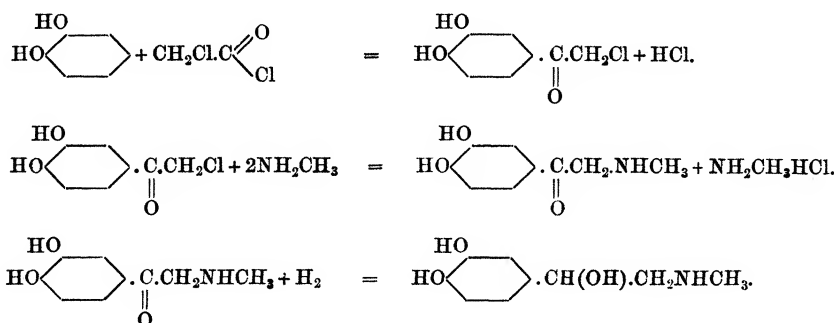
(c) On warming with aqueous alkali, methylamine is formed, so that the grouping, —NH.CH_3 is present.

(d) Adrenalin is optically active, and therefore contains an asymmetric carbon atom which will be situated in the side-chain.

results which would appear to exclude the second formula and support the first. Although criticism must be postponed until further particulars are available, still it may be noted that as at least one of the reactions—the formation of “peradrenalon”—appears to be abnormal, additional experimental evidence is very desirable.*

It is clear that the subject had reached a stage at which synthetical methods might be employed with a possible chance of success, and in January, 1904, I commenced experiments in this direction. A considerable part of my work has, however, been anticipated by workers in the laboratory of Meister Lucius and Brüning (9), and whilst I wish to disclaim any pretensions to priority, I take the opportunity of stating that my results were entirely independently arrived at, and that, owing to the method of publication adopted (Patent Specifications), it is only recently that I have become acquainted with the main portions of their work.

Dziergowski (10) has shown that when catechol is heated with chloracetylchloride, or with a mixture of chloracetic acid and phosphorus oxychloride, chloracetylcatechol is formed. It appeared probable that, by acting upon this substance with methylamine and subsequent reduction of the product, a substance would be produced which should have the formula (I) ascribed to adrenalin:—

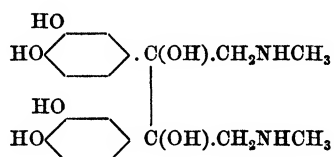


This series of reactions has been carried out, and a substance obtained which has very many of the physiological and chemical properties which are

* It may not be out of place to note that the statement made in abstracts of Friedmann's paper in the 'Biochemisches Centralblatt' and elsewhere to the effect that adrenalin on oxidation gives adrenalon (methylamino-acetyl-catechol) appear to be incorrect. This would obviously be of the utmost importance if true, but Friedmann apparently oxidised the benzenesulphonic acid derivative of adrenalin, and obtained a substance indistinguishable from the benzenesulphonic acid derivative of adrenalon, which is quite a different matter. As the substances are very badly characterised, too much weight must not be placed on the result, which might possibly be explained in other ways.

characteristic of adrenalin. It is true, further, that since the synthetic product would necessarily be optically inactive, one would expect slight differences between the artificial and natural bases, but on the other hand there are certain chemical differences, which will be mentioned later, which I am inclined to think are greater than would be the case if the two substances were stereo-isomeric. This involves the conclusion that either adrenalin or the synthetical base has *not* the structure represented by the formula $C_6H_3(OH)_2.CH(OH).CH_2NHCH_3$.

A substance which I believe to be identical with the base that I have prepared has been obtained by Meister Lucius and Brünig's chemists by the action of aluminium shavings and mercuric sulphate upon methylamino-acetylcatechol. They definitely ascribe the above formula to the substance, and this, if correct, would necessitate an alteration in the provisional structural formula of natural adrenalin. But it must be remembered that the synthetical base has been obtained by a reaction which is well known to give more than one product. Ketones commonly give a mixture of the corresponding secondary alcohol and pinacone upon reduction, either of which substances may constitute the main product. In the reaction under consideration the pinacone would have the following structure:—



Moreover, it must be remembered that the reaction is carried out, as will be seen later, under conditions which may quite well induce further changes in such unstable substances. Owing to experimental difficulties it is impossible at present to settle the point definitely, but I am of opinion that with the evidence at present available one is not justified in selecting preferentially any one of the several possible formulæ for the synthetical base.

The synthetical substance is extraordinarily active as regards the blood-pressure and certain other physiological effects.

Mr. T. R. Elliott was kind enough to examine some of the substance and informs me that so far as the experiments went they revealed no difference between the stimulating action—both motor and inhibitor—of the laboratory and of the animal products. Such close physiological resemblance may be taken as some evidence in favour of a close structural relationship between the two substances.

Experimental.

Chloracetylcatechol is prepared according to Dziergowski's (10) directions by heating catechol (1 mol.) and chloracetic acid (1 mol.) with phosphorus oxychloride equal in weight to the chloracetic acid used. As the reaction in my hands has always followed a course which differs slightly from that described by Dziergowski, the following particulars are given. The mixture should be placed in a large dry flask—at least 1000 c.c. capacity for 50 grammes catechol—and carefully heated in the water-bath. The mixture rapidly becomes fluid and clear, changing after a time to a deep purple colour. The reaction must be carefully watched, as at a certain point a violent evolution of hydrochloric acid with much frothing is liable to occur, which makes it necessary to cool the flask temporarily. Heating is continued until the evolution of hydrochloric acid slackens, and the thick dark-coloured semi-solid mass is dissolved in about three volumes of hot water. The solution is filtered to remove a trace of oily impurity and then allowed to cool. The chloracetylcatechol crystallises out in very well defined crystals, which are usually somewhat discoloured but are easily purified by a single re-crystallisation from boiling water. The yield is about equal in weight to the catechol taken. The dry substance must be handled with care, as although it is without smell, even minute traces of its dust have a very powerful irritant effect on the eyes and nose.

Methylamino-acetylcatechol.—The first product of the action of methylamine upon chloracetylcatechol is a yellow crystalline methylamine salt $(\text{CH}_3.\text{NH}_3.\text{O}.\text{C}_6\text{H}_3(\text{OH}).\text{CO}.\text{CH}_2\text{Cl})$ (10), but this substance on prolonged digestion with excess of methylamine gives methylamino-acetylcatechol, as has also been shown by Stolz (8). For example, 10 grammes of chloracetylcatechol is placed in a stoppered bottle and 25 c.c. of aqueous 33 per cent. methylamine added in portions of 5 c.c. each. The mixture is cooled between the successive additions. The ketone is almost immediately changed into the yellow crystalline salt, and after thorough shaking and allowing to stand for one or preferably two days, the mixture becomes dark brown from the separation of the crude base. The solid is filtered off and washed well with water, alcohol and ether, then shaken with a slight excess of dilute hydrochloric acid. The solution is filtered from any trace of unchanged chloroketone, concentrated and crystallised. Crystallisation is also economically effected by adding alcohol and subsequently ether to the concentrated aqueous solution, the hydrochloride being precipitated in the form of colourless crystals. The free base may be readily obtained from the hydrochloride by precipitating with ammonia and is, when pure, a colourless crystalline

substance, melting at 232° C. and is practically insoluble in water and neutral organic solvents. Most of the salts are readily soluble in water and give a beautifully crystalline compound when shaken with a strong solution of sodium bisulphite. The acetate forms a finely crystalline hydrazone on treatment with phenylhydrazine and a large excess of dilute acetic acid.

Reduction of Methylamino-acetylcatechol.—The reduction offered peculiar difficulties, since the majority of methods applicable for the reduction of ketones were inadmissible owing to the instability of these catechol derivatives in alkaline solution. However, it was found that reduction could be readily carried out electrolytically in acid solution using lead electrodes. The apparatus employed was similar to that devised by Tafel (11), and as it was important to keep the temperature of the liquid in the cathode compartment from rising above 16°, the cell was placed in a freezing mixture.

One part of the base to be reduced is dissolved in the minimum quantity of dilute hydrochloric acid, or preferably the crystalline hydrochloride is taken, and the solution diluted with 15 parts of water. Fifteen parts of 10-per-cent. sulphuric acid are then added to the solution, which is then placed in the cathode compartment. Owing to the sparing solubility of the sulphate of the base, it is advisable to add the sulphuric acid only just before commencing the reduction. The anode compartment is filled with 10-per-cent. sulphuric acid. The reduction is not very rapid, as the time required for reducing 1 gramme of base in a small apparatus, using a current of 3 to 4 ampères, is about half an hour. After the reaction is completed the bulk of the sulphuric acid is removed by adding baryta, and the filtrate contains the sulphate of the new base. A large number of modifications of the above reaction have been tried, but as the products appear to be identical and the above process is the simplest, it is not proposed to give further details, but it may be mentioned that the reduction of the bi-sulphite compound of the base is particularly promising. The aqueous solutions of the salts of the new base, however obtained, may be concentrated *in vacuo*, but are rapidly decomposed if heated on the water-bath.

On addition of ammonia, the free base is thrown out of solution as an amorphous greyish-white precipitate which very readily re-dissolves in acids. The base may be kept suspended in water for some little time without much change, but is so extraordinarily unstable in the dry condition that it has not yet been possible to obtain it in a fit condition for analysis. If one filters the base off as rapidly as possible and quickly removes adherent water by washing with dry alcohol and ether, it is found that the base has already undergone change, and is no longer completely soluble in dilute aqueous acids. This somewhat mysterious behaviour recalls that observed

by Victor Meyer (12) in the case of ω -aminoacetophenone ($C_6H_5.C:O.CH_2NH_2$), which readily forms stable salts, but the free base, when precipitated with ammonia, rapidly changes into a non-basic substance. It is mainly this peculiar property which leads one to conclude that it is unlikely that the synthetical base is the racemic modification of adrenalin, for the natural substance is quite stable in the dry state.

The salts of the base show the usual colour reactions with $FeCl_3$, etc., which are common to adrenalin and other catechol derivatives. Potassium ferrocyanide produces no precipitate in the cold, but gives a greenish precipitate on boiling. The free base, like adrenalin, is not precipitated on addition of sodium acetate to solutions of the salts, although under similar conditions the ketone base, methylamino-acetylcatechol, is precipitated. Picric acid produces no precipitate, but silver nitrate is, as one would expect, rapidly reduced.

If a solution of the sulphate be treated with the exact quantity of barium chloride necessary to remove the sulphuric acid and the solution be concentrated *in vacuo* at about 20° to 25° , a syrupy solution of the hydrochloride is obtained, but which shows no inclination to crystallise. The hydrochloride may be partly precipitated as an extremely deliquescent syrupy mass by adding anhydrous ether to a strong alcoholic solution.

The acetate is obtained in similar fashion from the sulphate by means of barium acetate. It has not been obtained crystalline, and seems to be more unstable than the other salts. On adding an alcoholic solution of oxalic acid to the acetate and then precipitating with excess of anhydrous ether, the oxalate is thrown out as a colourless hygroscopic oil which quickly shows distinct signs of crystallising, but it has not been possible to obtain satisfactory analyses for the salt.

A great many attempts were made to prepare stable crystalline derivatives suitable for thorough investigation, but as this end has not been obtained, it is not proposed to give any account of the products obtained by the action of substances such as benzoyl chloride, benzenesulphonic chloride, acetic anhydride, etc., as none of these derivatives were well characterised substances giving satisfactory analytical results.

Effect of Intravenous Injection of the Reduced Base.—The injection of extremely small quantities of the synthetical substance was found to produce a great rise in arterial pressure. A definite rise may be obtained when one millionth of a gramme of the base in the form of hydrochloride is injected into a rabbit with vagi divided. This and other physiological similarities noted by Mr. Elliott would seem to indicate that the difference between the natural and synthetical bases is but slight, and it is perhaps not inconceivable

that it is to some decomposition product which may be common to both substances that the stimulating property of the bases is due.

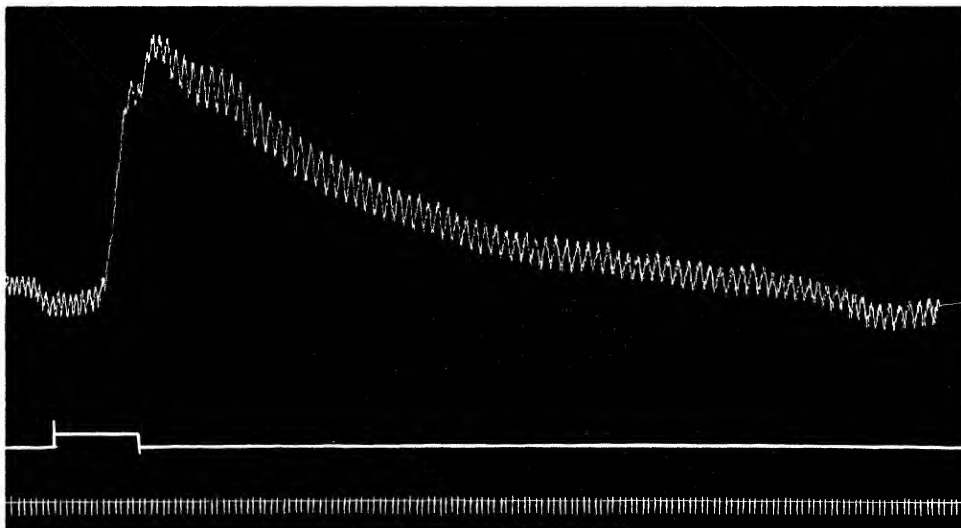


FIG. 1.—Rabbit, 3 kilos. Carotid B. P. Vagi divided. Urethane. Zero pressure 30 mm. below signal line. 0.00002 gramme base as hydrochloride. Time = seconds.

In conclusion I wish to acknowledge my indebtedness to Dr. Leathes for the very large amount of help and advice he has given me throughout the course of the work.

LITERATURE.

- (1) Takamine, 'Amer. Journ. Pharmacy,' vol. 73, November, 1901.
- (2) Aldrich, 'Amer. Journ. Physiol.,' vol. 5, p. 457, 1901.
- (3) Abel, 'Chem. Ber.,' vol. 36, p. 1839, 1903.
- (4) von Fürth, 'Sitzungsber. d. K. Akad. d. Wiss. in Wien,' vol. 112, Abt. III, 1903.
- (5) Friedmann, 'Beiträge z. Chem. Physiol. u. Path.,' vol. 6, p. 92, 1904.
- (6) Jowett, 'Trans. Chem. Soc.,' vol. 85, p. 192, 1904.
- (7) Pauly, 'Chem. Ber.,' vol. 36, p. 2944, 1903; and vol. 37, pp. 1388 to 1401, 1904.
- (8) Stolz, 'Chem. Ber.,' vol. 37, p. 4149, 1904.
- (9) D. R.-P., 157,300, Meister Lucius and Brüning.
- (10) Dziergowski, 'Journ. Russian Chem. Soc.,' vol. 25, p. 154.
- (11) Tafel, 'Chem. Ber.,' vol. 37, p. 3187, 1904.
- (12) Victor Meyer, 'Chem. Ber.,' vol. 28, p. 254, 1895.